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Biomarkers involved in cell transformation induced by nano-silica materials in the murine Bhas 42 model

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INTRODUCTION & OBJECTIVES

Synthetic amorphous silica nanomaterials (SAS) are used in many industrial sectors. However, we have previously found that SAS (namely, **NM-200**, **NM-202** and **NM-203**) led to cell transformation in the cell transforming assay developed by Sasaki et al (2015). Among them, **NM-203 was the most active** in triggering Bhas 42 cell transformation (Fontana et al., 2015) (Seidel et al., 2017).

In order to gain insight into the molecular mechanisms involved in the early stages of the SAS-induced cell transformation, we carried out a global and unsupervised transcriptomic analysis after a 48h-treatment with **NM-203**, **Min-U-Sil 5®** (a carcinogenic crystalline silica microparticle), **DE** (Diatomeous Earth, an amorphous silica microparticle) and **TPA** (12-*O*-Tetradecanoylphorbol-13-acetate, a tumor promoter) (**Table 1**).

Genes regulated by NM-203, Min-U-Sil 5®, and TPA-induced cell transformation were validated by RT-qPCR. Finally, we analyzed whether similar changes were also found after cell exposure to **NM-200 and NM-202**.

Sasaki K et al, J Environ Sci Health C Environ Carcinog Ecotoxicol Rev, 2015.

Fontana C et al, Mutat Res, 2017.

Seidel C et al, Nanotoxicology, 2017.

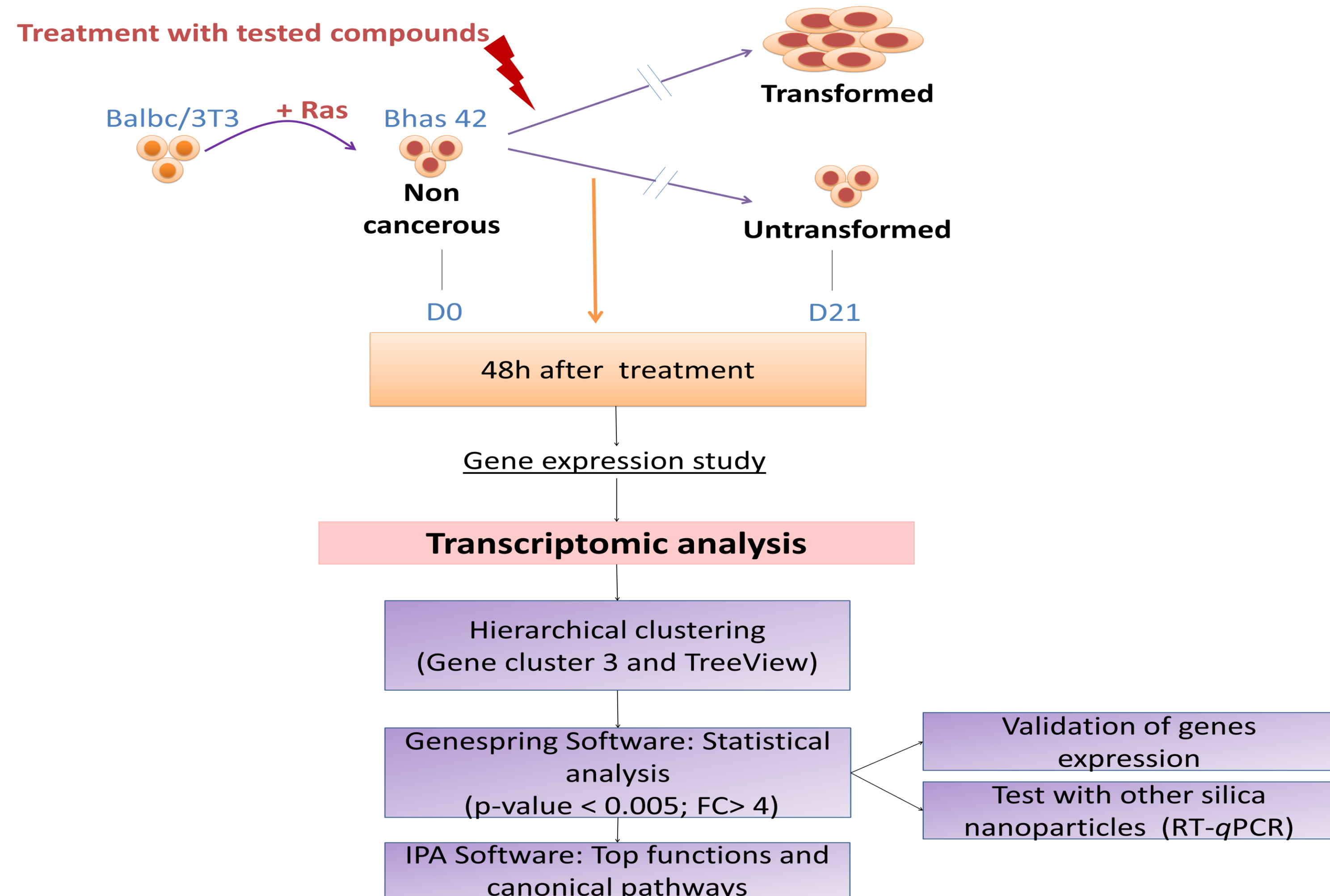


Figure 1: Bhas 42 cell transformation assay and experimental strategy: The Bhas 42 cell line is a fibroblastic murine cell line derived from Balbc / 3T3 cells transfected with the viral oncogene v-Ha-ras (Sasaki et al., 2015). Bhas 42 cells are then considered to be “initiated” cells. This cell line was used to study the effect of diverse SAS.

MATERIALS & METHODS

Table 1 : Physical characteristics of silica particles used in this study and cell transforming effect of compounds tested

| Silica/ Promoter agent | Bhas 42 assay | Main phase | Primary particle size | Impurities (wt%) | BET SSA (m ² /g) | DLS | |
|---------------------------|------------------|-------------|--------------------------|--|--------------------------------|----------------------------|-------------------------|
| | | | | | | Z-average in Water (nm) | Polydispersity index |
| NM-200 | + | Amorphous | 18.3 ± 4.5 nm | Al (0.87); Na (0.88); S (0.46) | 189 | ND | ND |
| NM-202 | + | Amorphous | 17.7 ± 2.5 nm | Al (0.45); Ca (0.18) | 204 | 232.8 ± 7.3 | 0.441 ± 0.012 |
| NM-203 | + | Amorphous | 24.7 ± 17.7 nm | Al (0.43); S (0.04) | 204 | 318.7 ± 4.9 | 0.423 ± 0.026 |
| Min-U-Sil 5® | + | Crystalline | 1.33 ± 0.85 µm | Al (0.16); Fe (0.05); Ca (0.04); Mg (0.01) | 5 | 1385.7 ± 377.0 | 0.318 ± 0.130 |
| DE | - | Amorphous | 1.35 ± 1.48 µm | Al (1.4); Fe (1.3); Ca (2.2); Ti (0.3); Mg (0.2) | 20 | ND | ND |
| TPA | + | | | | | | |

The Bhas 42 cell transformation assay was carried out as followed: Cells were seeded at high density and treated for 48 h with NM-203 at 1 and 2 µg/cm² (non cell transforming doses) and at 5 µg/cm² (a cell transforming dose), DE at 5 µg/cm², Min-U-Sil 5® at 20 µg/cm² and TPA at 0.05 µg/ml (**Figure 1**). Characteristics of tested chemicals are presented in Table 1.

RESULTS

Hierarchical clustering showed that non cell transforming treatments (NM-203 at 1 or 2 µg/cm², DE and controls) are closed and interestingly cell transforming treatments (NM-203 at 5 µg/cm², Min-U-Sil 5® and TPA) induced similar changes in gene expression (**Figure 2**). Several genes were **differentially expressed** after treatment with **NM-203, Min-U-Sil 5® and TPA**, while we did **not find any differentially expressed genes with DE**. Furthermore, IPA analysis showed that the main pathways common to NM-203, Min-U-Sil 5® and TPA treatments were involved in **cell proliferation**. Finally, twenty genes are common to all three conditions and among them, **twelve** relevant genes were selected (**Figure 3**). The expression of these genes was validated by RT-qPCR. In addition, deregulation of selected genes were also found with **NM-200 and NM-202** as shown for two selected genes POSTN (Periostin) and AUNIP (Aurora kinase A and ninein interacting protein) (**Figure 4**).

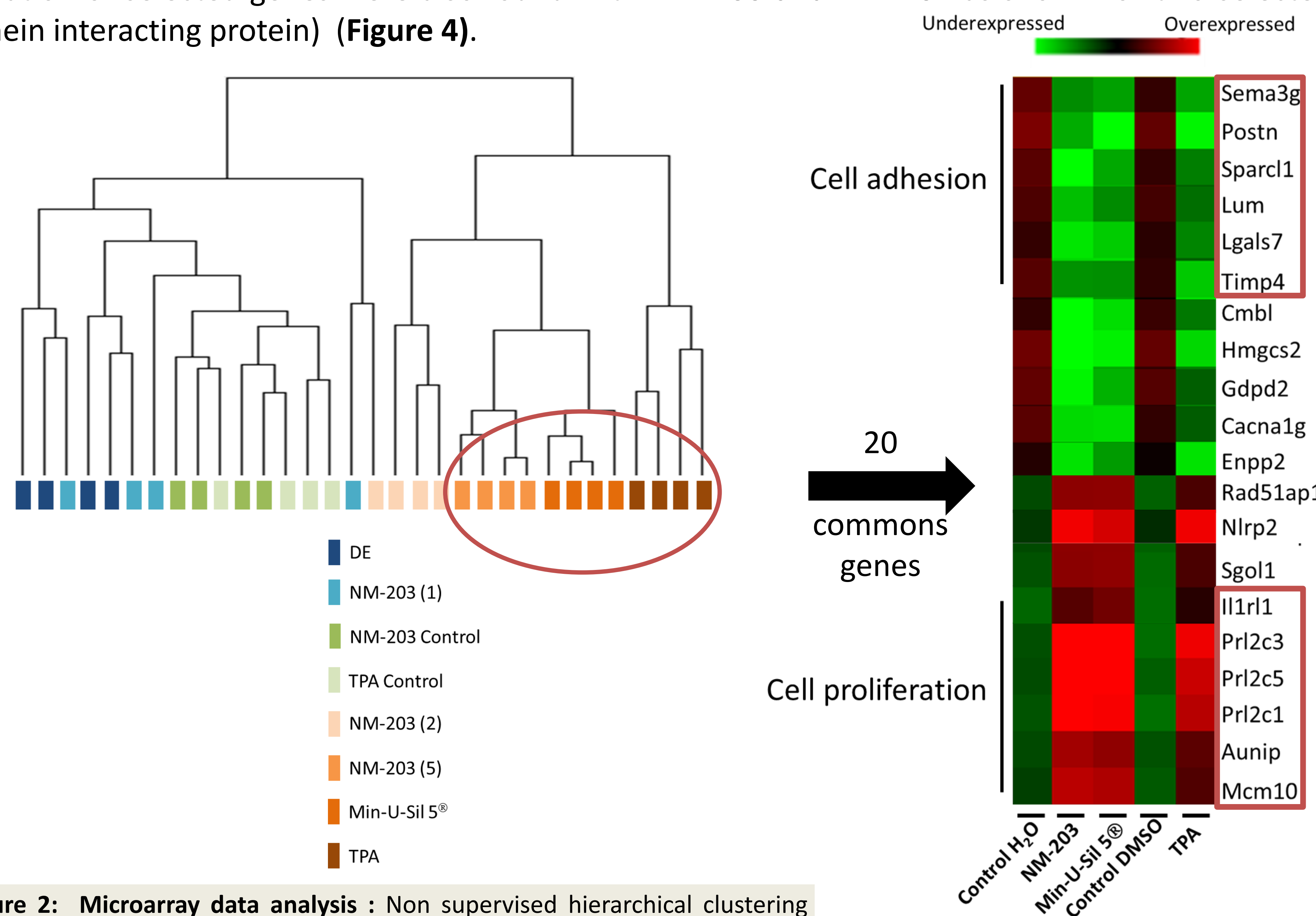


Figure 2: Microarray data analysis : Non supervised hierarchical clustering was obtained after filtration of microarray data (clustering was performed using a Gene Cluster 3 software and visualized with Treeview). Red circle show similar signature of three compounds NM-203, Min-U-Sil 5® and TPA.

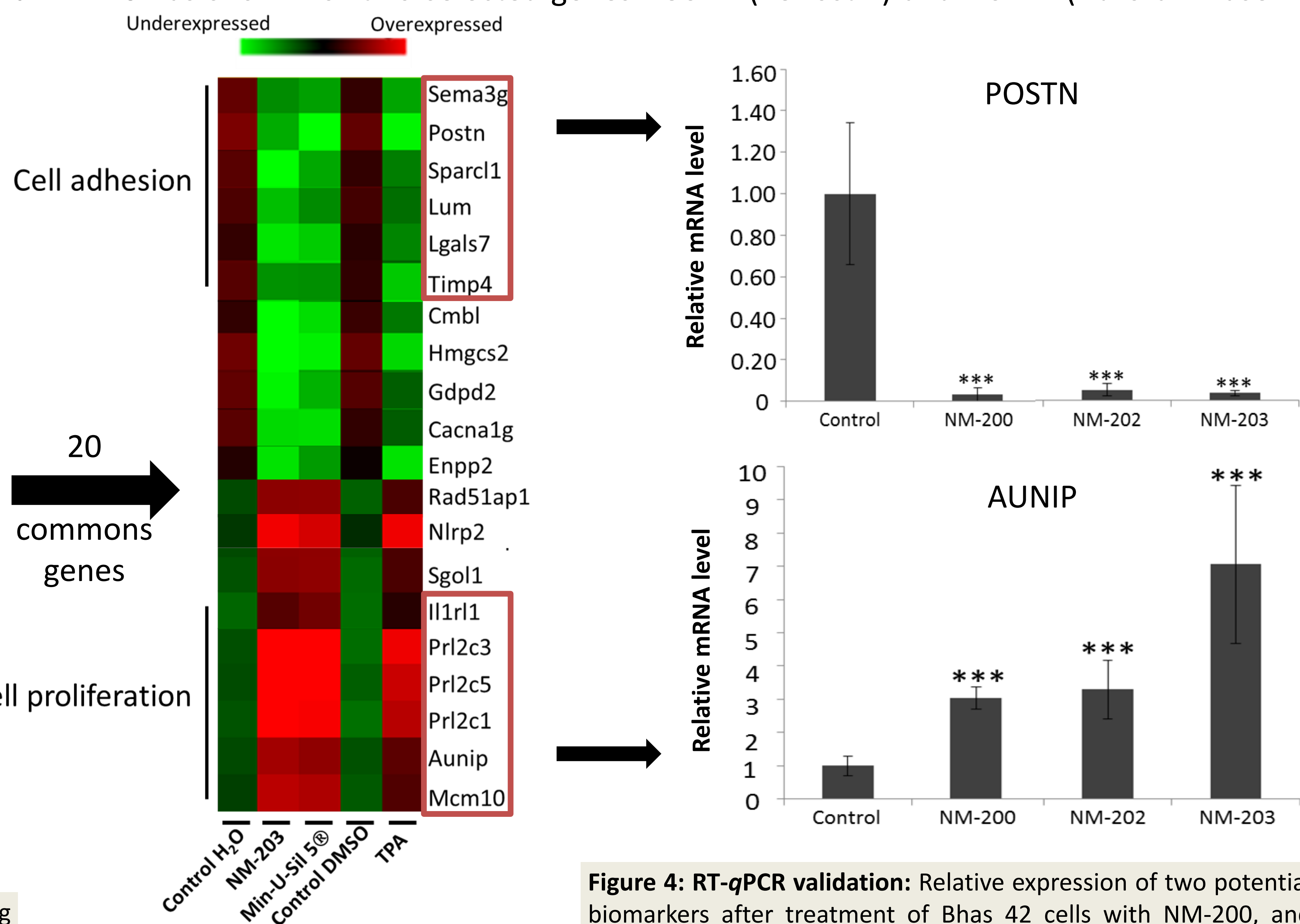


Figure 3: Heatmap of 20 common genes regulated by NM-203, Min-U-Sil 5® and TPA. p < 0.005; FC > 4

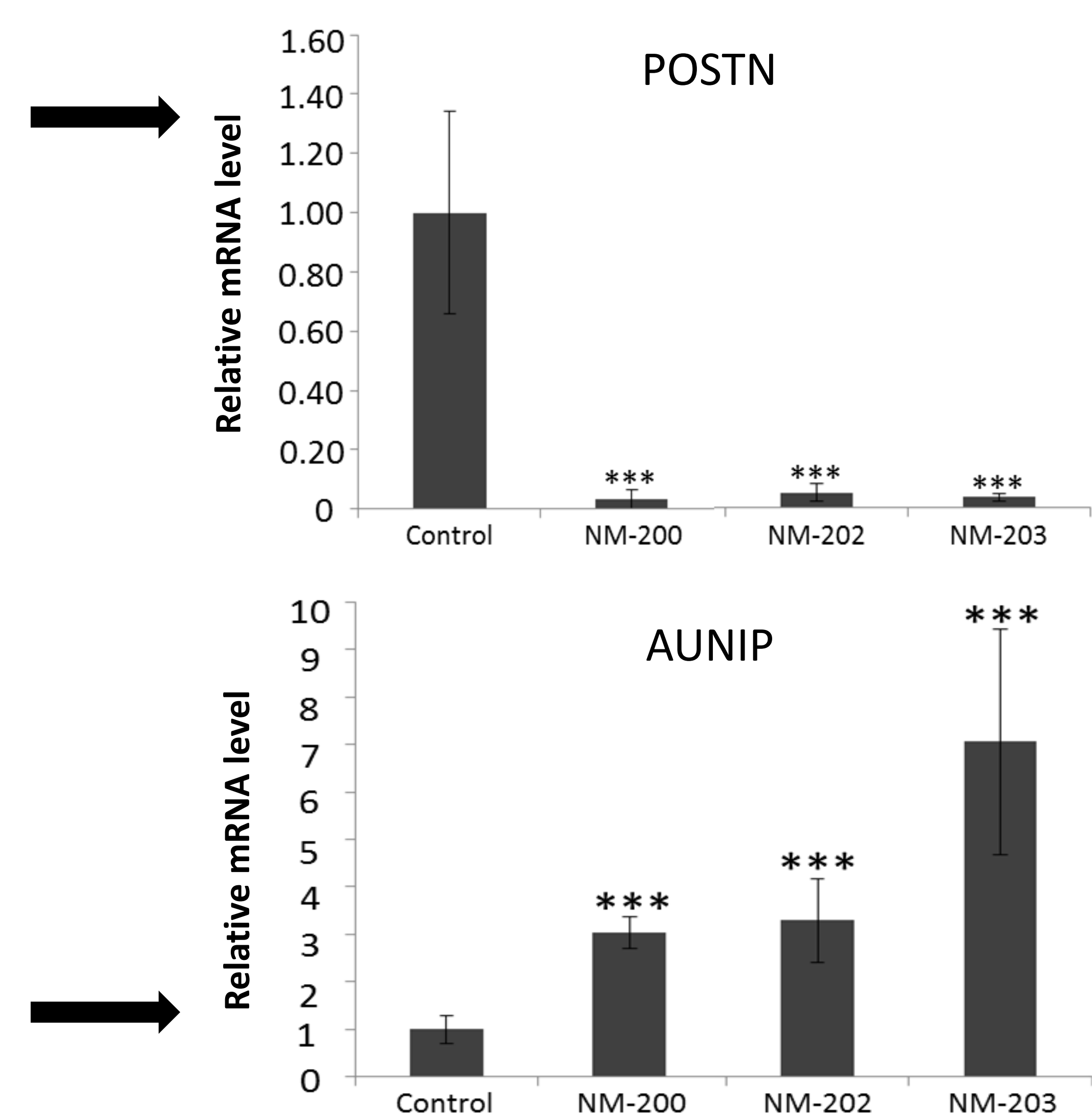


Figure 4: RT-qPCR validation: Relative expression of two potential biomarkers after treatment of Bhas 42 cells with NM-200, and NM-202. *** indicates p < 0.005 versus untreated cells after a mixed linear regression model followed by a Bonferroni multiple comparison test.

CONCLUSIONS

- ✓ For the first time, 20 common genes potentially involved in cell transformation were identified by a transcriptomic analysis after a 48h-treatment of Bhas 42 cells with **NM-203, Min-U-Sil 5® and TPA**.
- ✓ Deregulation of common genes has been found for two other SAS (**NM-200 and NM-202**).
- ✓ A set of 12 genes could be used as potential biomarkers of the early stages of Bhas 42 cell transformation for other SAS or other transforming nanomaterials.